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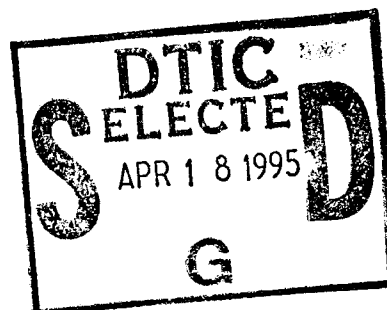Waterways Experiment
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Aquatic Plant Control Research Program

Methods for Monitoring Herbicide-Induced Stress in Submersed Aquatic Plants: A Review

by   Susan L. Sprecher, Michael D. Netherland

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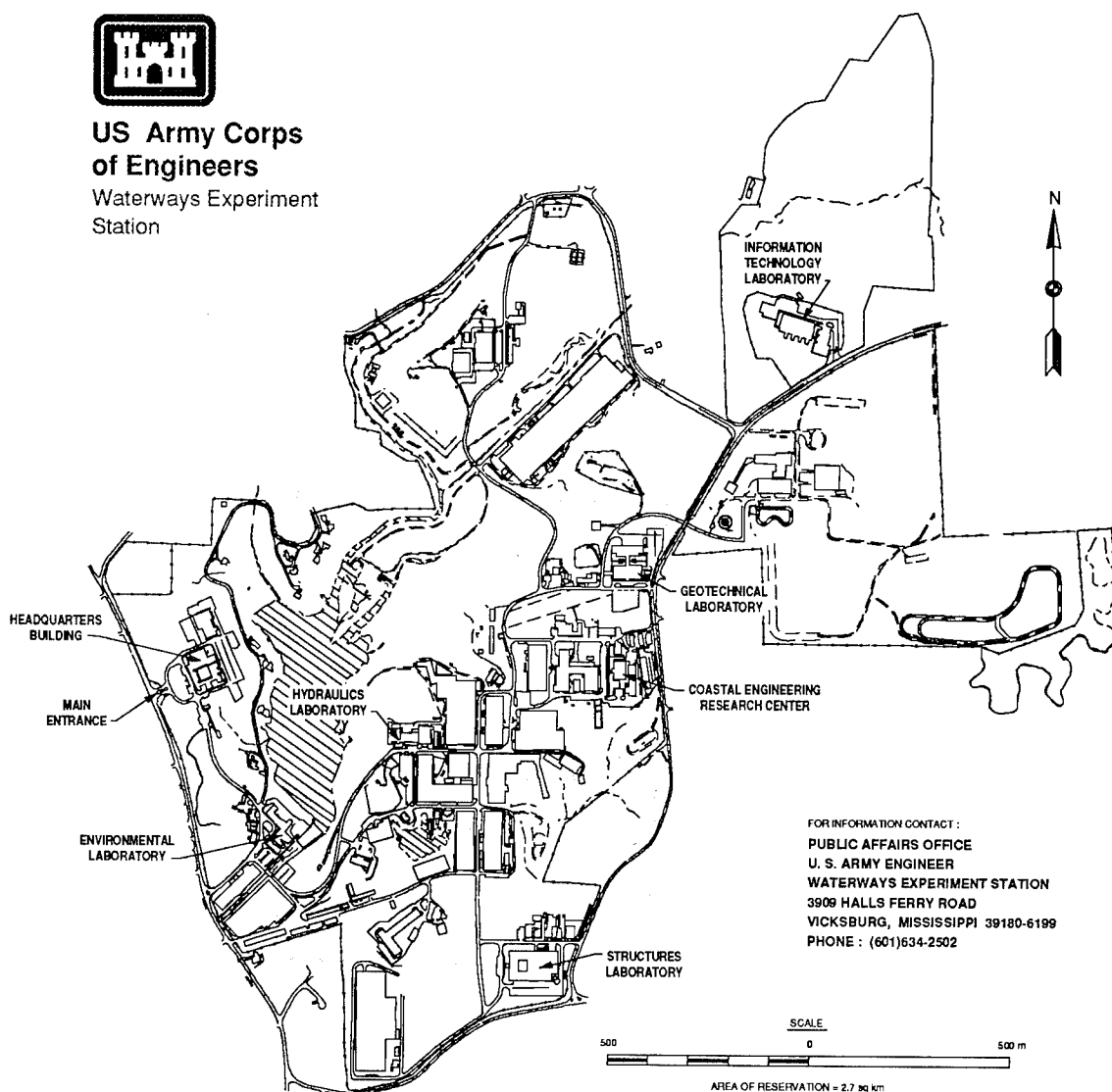3909 Halls Ferry Road
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Preface

The information reported herein summarizes a technical meeting entitled "Physiological Assessment of Herbicide Stress in Aquatic Plants," held 10 June 1993 as an effort of the Aquatic Plant Control Research Program (APCRP). The APCRP is sponsored by the Headquarters, U.S. Army Corps of Engineers (HQUSACE), and is assigned to the U.S. Army Engineer Waterways Experiment Station (WES) under the purview of the Environmental Laboratory (EL). Funding was provided under Department of the Army Appropriation No. 96x3122, Construction General. The APCRP is managed under the Environmental Resources Research and Assistance Programs (ERRAP), Mr. J. L. Decell, Manager. Mr. Robert C. Gunkel was Assistant Manager, ERRAP, for the APCRP. Technical Monitor at the time of this meeting was Ms. Denise White, HQUSACE.

The technical meeting summarized here was held to gather information on appropriate methods for investigating and documenting the physiological effects of aquatic herbicides on target (weedy) and nontarget submersed aquatic plants, in order to monitor the general and selective effects of these herbicides.

The report was prepared by Dr. Susan L. Sprecher, Research Biologist, and Mr. Michael D. Netherland, Biologist, Ecosystem Processes and Effects Branch (EPEB), Environmental Processes and Effects Division (EPED), EL, WES. Technical reviews of this report were provided by Dr. Kurt D. Getsinger, Research Biologist, and Ms. Linda S. Nelson, EPEB.

Scientists invited to participate in the technical meeting were Dr. Thomas Byl, U.S. Geological Survey; Dr. Stephen O. Duke, U.S. Department of Agriculture (USDA) Southern Weed Science Laboratory; Dr. Larry Dyck, Clemson University; Dr. Kien Luu, San Jose, CA; Dr. John H. Rodgers, Jr., the University of Mississippi; Dr. Donn G. Shilling, University of Florida; Dr. David F. Spencer, USDA Aquatic Weed Control Research Laboratory; Dr. Thai K. Van, USDA Aquatic Plant Laboratory; and Dr. William E. Spencer, Ecological Research Division, WES.

This technical meeting was held under the general supervision of Dr. John Harrison, Director, EL, and Mr. Donald L. Robey, Chief, (EPED), and under the direct supervision of Dr. Richard E. Price, Acting Chief, EPEB.

At the time of the publication of this report, Director of WES was Dr. Robert W. Whalin. Commander was COL Bruce K. Howard, EN.

This report should be cited as follows:

1 Introduction

Evaluation of aquatic herbicide efficacy on hydrilla (*Hydrilla verticillata* (L.f.) Royle) and Eurasian watermilfoil (*Myriophyllum spicatum* L.: hereafter called milfoil) has been a primary focus of Chemical Control Technology Team (CCTT) research at the U.S. Army Engineer Waterways Experiment Station (WES) for several years, and effective dosage rates for targeting these exotic weeds have been delimited (Green and Westerdahl 1990; Netherland, Green, and Getsinger 1991; Netherland and Getsinger 1992). While interest in concentration exposure time (CET) relationships for these weeds continues, research attention is now turning towards describing the impact of aquatic herbicides on a much broader range of plant species, with the goal of identifying application schedules and rates that can eliminate nuisance vegetation without imposing long-term injury on the desirable native and nontarget components of submersed aquatic plant communities. Availability of precise information on the interactions among herbicide, dose rates, and individual species will allow each of the relatively few compounds currently available for aquatic plant management to be used at its maximum potential for selective effect.

Additional research tools are being developed to support the CET delineations required to describe species-by-species herbicide effects. As well as acquiring information on life-cycle-related herbicide susceptibility through its phenology studies, the CCTT is exploring methods for monitoring physiological parameters that are able to verify and quantify herbicide effect in CET and selectivity research. Biomass measurements supported by visual evaluations remain the primary means of documenting herbicide efficacy and quantifying injury; however, they are inadequate when early, diagnostic and nondestructive evidence for response to herbicide treatment is required. In these cases, assays for physiological symptoms related to a herbicide's mode of action, or to the presence of metabolic stress, have several advantages. They can indicate onset and duration of effects of specific herbicides, at low treatment rates and before visual change occurs, with the use of only small amounts of tissue. Once identified, relatively quick and simple physiological assays related to each herbicide have potential for rapid screening of large numbers of species and treatment levels. Often the combined results from several such assays generate a more thorough understanding of metabolic changes associated with herbicide effect than can visual evaluation alone.

Physiological assessments are expected to be particularly useful in studying selective activity of herbicides, where the tolerance that a species exhibits to a compound may result either from absence of physiological response or from a dose-dependent response. Thus, monitoring for an internal change that is associated with herbicide effect can indicate whether a species lacks all response to a compound or develops susceptibility with increase in application rate. The former appears to be the situation with 2,4-D on most monocotyledonous species; the latter case has been observed with fluridone on sago pondweed (*Potamogeton pectinatus* L.), which tolerates a concentration of 2 $\mu g/\ell$, but shows injury when herbicide levels reach 15 $\mu g/\ell$ (unpublished data).

Although the CCTT is considering metabolic assays primarily as tools for laboratory and mesocosm tests, where limits on space, nutrients, and plant numbers preclude long-term observation and repeated destructive sampling, diagnostic tests and stress assessments also offer benefits in field situations. In the future, regulatory agencies may require proof that endangered or protected species in an aquatic community will not be adversely affected by proposed dosage rates of a treatment, or that they will emerge from a period of temporary application-induced stress with no long-term injury. Confirmation in such cases may be obtained by monitoring specific physiological processes known to be affected by the herbicide or general responses associated with plant stress. Lack of change in a diagnostic metabolite or the return of a stress-related compound to baseline levels then shows that plants were not affected or have recovered. Herbicide-specific diagnostic tests can indicate whether a compound has moved into off-target populations during application and can be used to monitor the effects of water retention or movement in these areas. In addition, such physiological tests can be used for verification or refutation in cases where low-dose or long-term exposure effects are suspected from terrestrial herbicide runoff.

CCTT scientists have associated changes in chlorophyll concentration (Westerdahl and Hall 1987; Netherland, Getsinger, and Turner 1993), photosynthesis and respiration (Netherland 1994), and enzyme activity (Sprecher, Stewart, and Brazil 1993a, 1993b) with herbicide effect in CET studies and are interested in utilizing additional plant stress monitoring techniques in laboratory, mesocosm, and field evaluations. Recently, a technical meeting on "Physiological Assessment of Herbicide Stress in Aquatic Plants" (see Appendix A) convened scientists from various universities and Government research facilities with common research interests in chemical control of plants and aquatic weed physiology and ecology. The discussion of stress assessment strategies and techniques for monitoring herbicide effects resulted in assay recommendations for the aquatic herbicides currently available. This report summarizes the general and specific assay guidelines recommended in the meeting and briefly describes the techniques that can be readily incorporated into the work of the CCTT.

2 Choosing and Using Physiological Assessments

The extensive literature that describes diagnostic mode of action assays for herbicidal compounds, bioassays for herbicide concentration quantification, and the use of plants for environmental toxicity and risk assessments, provides many procedures for evaluating the physiological effects of herbicides on plants (eg., Wang, Gorsuch, and Lower 1990; Gorsuch et al. 1991; Böger and Sandmann 1993; Dennison et al. 1993). These applications aim to identify a herbicide or concentration, or demonstrate that a specific metabolic pathway has been affected by a compound, or show that anthropogenic factors have produced a certain level of stress. In contrast, the CCTT's objective is to monitor herbicide-induced effects and efficacy and to quantify susceptibility or tolerance on specific plant species of interest. This difference in focus may require some modification in technique for published assays to be successful in evaluating the effects of known herbicides on submersed species. Discussions during the technical meeting produced some general guidelines on selecting and using physiological assays to accomplish this goal, and these are summarized here.

It was emphasized that change in a physiological parameter becomes increasingly informative and significant as it is related to change in growth. Reduction in growth or reproduction (reduction in fitness) remains the basic symptom of herbicide efficacy or effect on susceptible plants. When several plant functions are monitored and associated via multiple regression, the potential is increased for identifying correlations among metabolic processes and reduction in growth and for identifying those subsets of characteristics that can predict herbicide effect and efficacy. Multiparameter monitoring reflects the physiologically integrated responses with which a plant reacts to stress, whether herbicide induced (Duke and Kenyon 1986) or natural (Chapin 1991; Keeley 1991).

The efficiency of a parameter in indicating herbicide response should be verified in a small-scale experimental system before being applied to whole-plant setups or to the field. These initial systems can be either static, where growth is not expected and only physiological activity is monitored, or kinetic, where there is potential for growth-associated changes. The response of various physiological parameters to herbicidal compounds are commonly

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monitored via in vitro assays that use explants (eg., leaf disks or cotyledons) from terrestrial species or whole plants of the floating aquatic duckweed (*Lemna* spp.) as experimental units (eg., Duke, Kenyon, and Paul 1985; Cooley and Foy 1992; MacDonald, Shilling, and Bewick 1993). Comparable small-scale systems are in use for submersed species. Christopher and Bird (1992) and Bird (1993) monitored organogenesis in nodal cultures of milfoil to compare herbicide toxicity, and this technique is adaptable to other submersed species bearing whorls of leaves, such as hydrilla, elodea (*Elodea canadensis* Rich.), coontail (*Ceratophyllum demersum* L.) and egeria (*Egeria densa* Planch.). Flask culture of unrooted apical portions or rooted plants in test tubes or jars provide larger experimental units for aquatics (Van and Conant 1988; Hinman and Klaine 1992; Netherland and Lembi 1992) and are readily adapted to monitoring samples collected from the field following treatment.

There are a few caveats related to these tests. The system chosen needs to be suited to the herbicide's mode of action and the species' growth habit. For example, a leaf disk system cannot be used to monitor decreased levels of chlorophyll following fluridone treatment since the diagnostic bleaching effect of this herbicide is only seen in newly emerging tissue. And while some parameters may be adequately monitored by using explant procedures alone, in cases where groups of assays are to be correlated to growth of the whole plant, tests need to be conducted on vegetation undergoing a normal life-cycle in aquaria, mesocosms, or the field.

A parameter that changes as a direct result of a compound's mode of action is valuable as a diagnostic test for the presence of a specific herbicide and may allow dose-related quantification of effect. For example, enzymatic metabolism of shikimate is prevented by glyphosate. Increase in shikimate levels with treatment, and decrease with cessation of treatment, is diagnostic for this herbicide. Monitoring a diagnostic compound may make it possible to pinpoint a threshold dose at which metabolic change is produced or to verify that a nontarget species is immune to effect, as well as to exclude variability in response arising from environmental factors. However, diagnostic responses can require complex analysis and are not always the most efficient way to monitor treatment effect. An indirect response able to be measured rapidly and accurately, and known to change predictably with treatment, may serve as well. As an example, change in chlorophyll fluorescence is not a direct effect of glyphosate's mode of action, but it is closely associated with the herbicide's activity and is much easier to monitor as a symptom of treatment than is shikimate level.

A metabolite that is induced or accumulates to high levels because of herbicide treatment is more useful as an indicator of effect than one that changes less dramatically. This is because an increase from an initial zero or negligible background level of a compound is easier to measure than a small incremental change in a pre-existing substance. In normally growing plants, the carotenoid precursor phytoene is essentially absent; it accumulates in emerging tissues following fluridone application. This qualitative difference makes phytoene readily associated with treatment effect.

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Physiological assessments tend to be more reliable in mature tissue than in younger plant parts since normal physiological changes involved in development may cause ambiguity in the expression of some parameters. However, where herbicides primarily affect physiological development in young tissue, as is the case with the compounds fluridone and bensulfuron methyl, assays that target these plant parts are needed.

Finally, prepackaged assays or test kits for a variety of scientific and environmental monitoring are widely available from commercial sources. The convenience, standardization, and repeatability of assays in kit form may be particularly useful in the field. Packaged assays for carbohydrate and total protein determinations are examples of tests that are applicable to evaluating herbicide effects. Other relevant assays should be identified and investigated.

3 Assays for Physiological Effects of Aquatic Herbicides

The commonly used aquatic herbicides, including those that have recently been or are being pursued for registration, are listed below. They are grouped by their mode of action, and each is followed by physiological parameters suggested by meeting participants as being diagnostic or especially suitable to CCTT evaluations because of ease of measurement. These parameters and tests are summarized in the final section of this paper.

Pigment Inhibitors - Fluridone

The effect of fluridone (1-methyl-3-phenyl-5[3-(trifluoromethyl)]-4(1H)-pyridinone) on susceptible plants is primarily on newly emerging tissue, which is prevented from synthesizing carotenoids because of inhibition of phytoene desaturase in the carotenoid biosynthetic pathway (Bartels and Watson 1978; Sandmann and Böger 1989). Without the protection of these pigments, chlorophyll is photooxidized in sunlight. Newly emerging tissues appear bleached and photosynthesis is inhibited in them, although previously mature leaves remain green and photosynthetically active. Pigment inhibition is present as long as the chemical is present; once the herbicide is removed, new tissue is able to develop normally, and plants may grow out of damage if initial exposure has been insufficient. Although symptoms are manifested within 3 to 6 days, control and death occur over a period of weeks as the plant depletes its reserves of assimilates (Arnold 1979; McCowen et al. 1979).

A range of low dosages (5 to 40 μg/ℓ) of fluridone are adequate for control of hydrilla and milfoil if exposure can be prolonged (60 to 90 days) (Hall, Westerdahl, and Stewart 1984; Netherland, Getsinger, and Turner 1993; Netherland 1994). CCTT laboratory studies have shown that physiological changes occur in hydrilla at treatment rates as low as 1 μg/ℓ (Netherland 1994). Although visual symptoms such as chlorophyll degradation are essentially nondetectable for several weeks, changes in photosynthetic rates (monitored as oxygen evolution) are manifested within days; both have been

related to dose and amount of control in hydrilla and milfoil (Westerdahl and Hall 1987; Netherland 1994).

Most of the following tests, suggested in the technical meeting as suitable for monitoring fluridone, are appropriately monitored in emerging tissue that is being affected by the compound. However, comparison of new tissue with mature material may indicate the modifications caused by the herbicide and the cumulative effect on the whole plant.

Phytoene. Inhibition of phytoene desaturase is characterized by accumulation of carotene precursors to levels significantly above the negligible amounts seen in untreated plants (Sandmann and Böger 1983), so that the presence of phytoene in newly emerged tissue provides a unique diagnostic symptom of fluridone effect. It is possible that this property may be used to reveal herbicide contact in plants that do not exhibit marked chlorosis or other visual changes with treatment.

Carotene. Synthesis of carotenoids is prevented in new growth, and reduced levels of ß-carotene in apical tips and newly emerged leaves are expected in treated plants.

Chlorophyll. The degree of chlorosis produced by fluridone in emerging tissues has been shown to be dose proportional in several species. The regularity of the chlorophyll degradation exhibited by aquatic charophytes has indicated their use as a bioassay for this herbicide (Burkhart and Stross 1990).

Conductivity. Measured in newly bleached tissue or in chlorophyllous portions that matured prior to treatment, conductivity indicates status of membrane integrity in the plant.

Biomass/growth. Changes in levels of stored carbohydrate and in rate of carbohydrate assimilation, as well as in the rate of production of new tissue, are measures of treatment effect that can be related to regrowth potential. Tuber and turion production in hydrilla has been shown to be affected by fluridone (MacDonald et al. 1993).

Contact Herbicides

Contact herbicides cause injury to the tissues they contact and are not appreciably translocated in plants. They are primarily membrane and cell disruptors and are effective following short exposures (4 to 24 hr) of relatively high doses (2 mg/ℓ). Symptoms appear soon after treatment, and the onset of plant death can be rapid. Although general mechanisms of activity are similar, appropriate assessments differ with each herbicide and its mode of action. The effect of longer term (3- to 10-day) exposure to threshold concentrations of contact herbicides has not been adequately investigated to support minimum dosage applications in the field, and information on low-dose effects at the physiological level is particularly lacking.

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Copper complexes

Low application rates of 0.2 to 1.0 mg/ℓ metallic copper produce visible effects in 1 to 10 days. The copper complexes are membrane disruptors and cause photosynthetic inhibition through binding to chloroplast membranes, disrupting electron transport in Photosystem II (Weed Science Society of America (WSSA) 1989). Where copper is used as an algicide, it may be necessary to monitor any detrimental effect on nontarget macrophytes.

Chlorophyll *a* fluorescence. Increases early in treatment because of loss of light-harvesting function of membranes in Photosystem II.

Oxygen uptake/evolution. Uptake decreases because of breakdown in photosynthetic ability; respiration is not expected to change.

Oxidative enzyme activity. Increased levels of peroxidase have been seen in shoot but not root tissue with copper stress in hydrilla (Byl and Klaine 1991).

Conductivity. Variable; changes as membrane activity is disrupted. Can be related to increase in chlorophyll *a* fluorescence.

Diquat

Within the photosynthetic pathway, diquat (6,7-dihydrodipyrido[1,2-α:2′,1′-*c*]pyrazinediiium ion) cations accept electrons from Photosystem I and become active free radicals. These indirectly cause the production of superoxide radicals that rapidly disrupt cell membranes via lipid peroxidation (WSSA 1989). The action of this photobleaching herbicide is more rapid in light than dark.

Rate of herbicide uptake varies by species; contact times from 6 to 48 hr gave control of hydrilla with concentrations ranging from 2.0 to 0.25 mg/ℓ (Van and Conant 1988). Susceptible species decline rapidly, often within 7 days. The reduction of colorless tetrazolium hydrochloride to a colored form in living cells has been used as a test of viability following diquat treatment of elodea (Davies and Seaman 1968). Cassidy and Rodgers (1989) found dissolved oxygen (uptake and evolution) and membrane permeability (conductivity) to be sensitive to diquat treatment in hydrilla.

Chlorophyll *a* fluorescence. Loss of light energy to fluorescence occurs as the integrity of chloroplast membranes is disrupted. MacDonald, Shilling, and Bewick (1993) used the peak/terminal ratio in measuring this parameter and saw fluorescence increase with diquat treatment.

Oxygen uptake/evolution. Evolution in light decreases sharply as photosynthetic function is impaired (Cassidy and Rodgers 1989). Oxygen

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consumption in the dark rises rapidly as the respiration rate increases (MacDonald, Shilling, and Bewick 1993).

Conductivity. Conductivity increases rapidly (1 to 4 hr) as membranes are disrupted and leakage occurs; it is correlated to treatment dose (Cassidy and Rodgers 1989; MacDonald, Shilling, and Bewick 1993).

Cytochrome *f*. Cytochome *f* has been used to detect effects of paraquat (1,1'-dimethyl-4-4'-bibyridinium ion), which is similar in action to diquat. Paraquat produces an increase in the rate of the dark-induced change to the reduced form of cytochrome *f* (Vaughn and Duke 1983).

Oxidative enzyme activity. The free radical formation induced by paraquat and diquat produce oxidative stress and are expected to increase activity of enzymatic antioxidative defenses such as peroxidases, catalase, and superoxide dismutase (Scandalios 1993).

Tissue burden. Measurement of diquat in plant tissue and surrounding water following treatment has been used in monitoring the herbicide's effect on hydrilla and correlated to conductivity and oxygen evolution (Cassidy and Rodgers 1989).

Endothall

This contact type, membrane-active, herbicide (7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic acid) inhibits protein synthesis upon being metabolized by plants and is selectively effective. There is some translocation in aquatic plants, although rate of initial uptake is slow. A range of concentration/exposure times, from 5 mg/ℓ for 12 to 24 hr, to 0.5 mg/ℓ for 72 hr, has been found to be effective on hydrilla and milfoil (Netherland, Green, and Getsinger 1991). Among the contact herbicides mentioned here, endothall does not directly produce a change in chlorophyll *a* fluorescence (MacDonald, Shilling, and Bewick 1993).

Oxygen uptake/evolution. Evolution in light increases temporarily, while oxygen consumption in the dark decreases, as the ability of tissue to use oxygen is reduced (MacDonald, Shilling, and Bewick 1993).

Conductivity. This parameter increases rapidly and is very predictive of injury, using total potential leakage or percent conductivity as a basis for comparison (MacDonald, Shilling, and Bewick 1993).

Oxidative enzyme activity. Peroxidase was seen to increase in hydrilla, which is controlled with treatment by the dipotassium salt formulation of endothall, while levels in the tolerant species *E. densa* remained unchanged (Sprecher, Stewart, and Brazil 1993).

Growth Regulator Herbicides - 2,4-D and Triclopyr

2,4-D - (2,4-dichlorophenoxy)acetic acid and triclopyr - [(3,5,6-trichloro-2-pyridinyl)oxy]acetic acid are systemic compounds that mimic the plant hormone auxin, causing excessive growth and cell division (WSSA 1989). Low doses can be effective if exposure time is moderate; short exposure times are effective with higher doses. However, high doses may cause such rapid injury that potential translocation throughout the plant is prevented. Symptoms of epinasty and leaf curling are manifested rapidly (1 to 2 days) even at very low doses, but plant death is slower. Visible effects on plant growth may be seen at levels far below those required for control, and physiological symptoms may provide more predictive indicators of efficacy than visual evaluations at early posttreatment stages.

In milfoil and hydrilla, doses of 0.25 to 0.5 mg/ℓ for 3 to 5 days, or 8- to 24-hr exposure times with higher doses, 1.0 to 2.5 mg/ℓ, provide good control (Green and Westerdahl 1990; Netherland and Getsinger 1992).

Ethylene. Increase in evolution of this gas is a sensitive, early response to these compounds and is correlated to dose (eg., Hall et al. 1985).

Total protein/nucleic acids. Treatment-induced increases in cell division stimulate ribonucleic and deoxyribonucleic acid (RNA and DNA) production and protein synthesis in stems of susceptible plants. Levels of nucleic acids increase in about 24 hr. This response is unique to the growth regulator herbicides. However, high concentrations of herbicide in shoot and root tips can suppress synthesis of protein and nucleic acids in sensitive plants.

Oxygen uptake/evolution. Respiration increases initially (WSSA 1989).

Conductivity. Increases on both susceptible and tolerant species, with tolerant plants later recovering pretreatment levels.

Oxidative enzyme activity. Milfoil has been seen to increase peroxidase enzyme activity with triclopyr treatment (Sprecher and Stewart, unpublished data).

Tissue burden. The systemic nature of these compounds may make this a useful parameter to monitor. However, initial tissue burden results from field work suggest that it shows a wide range of variability in measurement.

Amino Acid Inhibitors

Shikimic acid pathway disruption: Glyphosate

Glyphosate's (N-(phosphonomethyl)glycine) main mode of action is inhibition of the shikimate pathway as glyphosate competes with the substrate phosphoenolpyruvate (PEP) for binding to 5-enolpyruvylshikimate-3-phosphate synthase (EPSP synthase). Glyphosate disrupts biosynthesis of the aromatic amino acids phenylalanine, tyrosine, and tryptophan from shikimate (WSSA 1989). Generally, results in slow plant death (14 days or longer) as loss of ability to assimilate carbohydrates through this important biosynthetic pathway eventually depletes the plant's reserves. Current aquatic use is only on floating or emergent and wetland vegetation; glyphosate is not effective for control of submersed species.

Shikimate/benzoate levels. The marked accumulation of the EPSP synthase substrate shikimate and of hydroxybenzoic acids is diagnostic for glyphosate effect and can be identified by high performance liquid chromatography (HPLC) (Hoagland and Duke 1982).

PAL levels. Substantial increase in extractable phenylalanine ammonia lyase (PAL) in response to decrease in levels of its amino acid substrate phenylalanine. This response, although indirect, is also diagnostic for glyphosate effect (Hoagland and Duke 1982). PAL can be quantified, following precipitation from crude plant extracts, by a reaction with phenylalanine. The resulting cinnamic acid production is monitored spectrophotometrically (Duke and Naylor 1976).

Chlorophyll *a* fluorescence. Although this photosynthetic effect is an indirect response to glyphosate treatment, it occurs soon (30 min to 2 hr) after treatment[1] and is simple to measure in the laboratory or field (Bolhàr-Nordenkampf et al. 1989).

Carbon exchange. CO_2 uptake slows as photosynthesis decreases, and the depletion of carbon compounds eventually results in death.

Oxygen uptake/evolution. Respiration rates increase while photosynthesis decreases.

Biomass/growth. Carbohydrate reserves are depleted, and assimilation and growth is reduced.

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[1]   Personal Communication, 10 June 1993, Dr. Stephen O. Duke, Plant Physiologist, USDA-ARS Southern Weed Science Laboratory, Stoneville MS.

ALS inhibitors: Sulfonylureas, imidazolinones, sulfonamides

Inhibition of acetolactate synthase (ALS; also known as acetohydroxyacid synthase, AHAS) by these compounds prevents incorporation of pyruvate into branched-chain amino acids in both the isoleucine and valine/leucine biosynthetic pathways, resulting in inhibition of cell division in growing tips of roots and shoots (Beyer et al. 1988). Symptoms appear in 1 to 7 days, and death occurs slowly over a period of weeks as protein synthesis is reduced and growth is inhibited.

Bensulfuron methyl (methyl 2-[[[[[(4,6-dimethoxy-2-pyrimidinyl)amino] carbonyl]amino]sulfonyl]methyl]benzoate) has been shown to inhibit ALS activity in hydrilla (Rattray et al. 1993) and is active at very low rates on a number of aquatic plants (Anderson and Dechoretz 1988). Doses of 50 to 150 $\mu g/\ell$ have been found to be effective in controlling milfoil provided an adequate exposure is maintained (45 to 60 days) (Nelson, Netherland, and Getsinger 1993). Bensulfuron methyl also inhibits growth and tuber formation in hydrilla (Van and Vandiver 1992; Haller, Fox, and Hanlon 1992; Langeland and Laroche 1992). Although this compound is no longer being considered for noncrop aquatic registration, the high biological activity and frequent agricultural use (eg., for rice) of this and similar sulfonylureas and imidazolinones suggest that the ability to determine their effects on nontarget aquatic plants is useful.

Amino acids. Levels of leucine, isoleucine, and valine decrease with ALS inhibitors. Reversal of this effect with removal of treatment is diagnostic for these herbicides. Branch chain amino acids are identified chromatographically, and automated amino acid analysis is available (Rhodes et al. 1987; Cooley and Foy 1992).

α-ketobutyrate derivatives. α-ketobutyrate is a substrate of ALS in the synthesis of isoleucine, and increased levels of its alternate derivatives (2-oxobutyrate, α-aminobutyrate) occur when the normal amino acid synthetic pathway is blocked by ALS inhibitors (Rhodes et al. 1987). These derivatives are monitored with gas chromatography.

Nucleic acids. The rate of nucleic acid production decreases rapidly in an indirect response to treatment, as the stage of cell division in which DNA is synthesized is prevented from occurring (Beyer et al. 1988). Reddish brown discoloration of apical tips and shoots is caused by accumulation of anthocyanins and phenolic compounds during this inhibition of cell division (Beyer et al. 1988), and disappearance of these pigments with removal of treatment is also diagnostic.

Oxidative enzyme activity. Increase in peroxidase activity was found to be dose dependent following treatment of hydrilla with the sulfonylurea herbicide sulfometuron methyl (methyl 2-[[[[94,6-dimethyl-2-pyrimidinyl)amino]-carbonyl]-amino]sulfonyl]benzoate) by Byl and Klaine (1991).

Biomass/growth. Shoot and root elongation decreases in susceptible plants (Langeland and Laroche 1992), and tuber production in hydrilla is suppressed (Van and Vandiver 1992; Haller, Fox, and Hanlon 1992). Growth inhibition of shoots was found to be a more consistent estimate of herbicidal efficacy than dry weight in hydrilla (Rattray et al. 1993).

Photosystem Inhibitors - Triazines

In general, these photosynthetic inhibitors prevent oxygen evolution, electron transport, and carbon fixation, thereby inhibiting synthesis of starch. Binding to the D-1 (quinone-binding) pigment protein of Photosystem II by these herbicides blocks electron flow through the light reaction portion of photosynthesis (Duke 1990). The accompanying damage to membranes is thought to be a secondary effect because of lipid peroxidation caused by free radicals (WSSA 1989). Similar to copper in herbicidal activity, a wide range of doses requires long exposure to give control. Symptoms appear quickly, within 1 to 7 days, but plant death is slow.

Although simazine (2-chloro-4,6-bis(ethylamino)-s-triazine) is not being reregistered for aquatic use, runoff of the related herbicide atrazine (6-chloro$N$,N'-diethyl-1,3,5-triazine-2,4-diamine) has been blamed for plant declines in some aquatic systems. Diagnostic tests for its effect may have application in regulatory and mitigation situations.

Chlorophyll *a* fluorescence. Increase in the fast (initial) phase of fluorescent emission occurs immediately after treatment as electron-retention capacity by Photosystem II is lost through herbicide binding. The diagnostic emission pattern produced by atrazine-type herbicides results from an increase in fluorescence from normal levels in terrestrial plants (Miles 1990; MacDonald, Shilling, and Bewick 1993).

Oxygen uptake/evolution. Oxygen evolution in light decreases sharply as the rate of photosynthesis declines within a few hours of treatment. In tolerant plants, the rate of photosynthesis decreases less than in sensitive plants and returns to normal within a few days, while in susceptible plants the rate drops to near zero within 1 or 2 days and does not recover. Respiration does not change.

Conductivity. Cellular leakage occurs because of membrane damage caused by lipid peroxidation.

Oxidative enzyme activity. Increase in peroxidase activity has been seen with atrazine treatment levels of 0.1 to 1.0 mg/ℓ.[1]

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[1] Personal Communication, 10 June 1993, Dr. Thomas Byl, Plant Physiologist, U.S. Geological Survey, Nashville TN.

Cytochrome *f*. Change in the oxidation/reduction cycle of this photosynthetic compound has been measured in vivo in terrestrial plants to detect effects of the related herbicide atrazine (Vaughn and Duke 1983; Duke 1993).

Tissue burden. Enzyme-linked immunosorbent assay (ELISA) allows monitoring of amounts of herbicide taken up by the plant. Hinman and Klaine (1992) used [^{14}C] atrazine to show uptake and translocation in rooted hydrilla.

4   Assays and Diagnostic Tests

The following section gives brief summaries and references for those assay parameters that are appropriate for incorporation into the CCTT's herbicide evaluations. Most of the protocols cited require only currently or readily available laboratory equipment (clinical centrifuge, autoclave, spectrophotometer, etc.), although procedures may need modification when applied to a particular species or tissue. A few of the assays can be field adapted (eg., chlorophyll fluorescence), while others are suitable to field sampling with subsequent laboratory analysis.

Biomass/Growth

Quantification of biomass, the weight of living plant matter, is the parameter most commonly used to demonstrate effects of herbicide treatment on growth and vegetative reproduction in aquatic plants. Dry weight biomass of plant material oven-dried to a constant weight is a standard and rapid laboratory assessment. As with yield in agricultural systems (see Evans 1972), the quantification of metabolic productivity and growth in aquatic plants can be assessed by monitoring change in number and/or size of parts, eg., leaf area, plant height, shoot length, and number of nodes or propagules, along with rates and ratios based on these data (eg., Grace and Wetzel 1978). When plant parts are sampled separately, biomass data can reveal whether treatment produces change in resource allocation or reproductive strategy. Ratios of emergent to submersed vegetation or comparison among rhizomes, tubers, turions, and seeds have been used with aquatic plants (Sytsma and Anderson 1993; Luu and Getsinger 1990). Nutrient levels within tissues, particularly the macronutrients nitrogen and phosphorous, also provide estimations of growth (Nichols and Keeney 1976; Sytsma and Anderson 1993).

Biomass sampling in the field is labor intensive (often underwater), but provides essential information on herbicide effect on target and nontarget species (Sliger, Henson, and Shadden 1990; Madsen 1993). It is facilitated by experimental design that provides for the most efficient use of sampling transects and quadrats to give valid statistical analysis (Downing and Anderson

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1985; Madsen 1993; Titus 1993). Techniques such as aerial imaging (Farone and McNabb 1993) and echosounding (Madsen 1993) furnish estimates of biomass, but cannot show the changes in species composition that are the goal of selective herbicide use.

Since some submersed aquatic plants have the capacity to regrow from root crowns or rhizomes, or to root from stem fragments, it is often desirable to monitor regrowth after cessation of herbicide treatment. This indicates whether regeneration from crowns or underground structures is possible, or whether permanent control has been achieved (eg., Nelson, Netherland, and Getsinger 1993).

Carbohydrate Content

In a stressed plant, levels of starches or sugars may be depleted as assimilate reserves are used and not resupplied. Carbohydrate status can be measured rapidly in the laboratory on large numbers of fresh or dried samples, and this parameter is particularly applicable where exposure time is long and herbicide effects are not immediately evident. Total nonstructural carbohydrates or starch alone can be monitored, using colorimetric methods (Nelson 1944; Dubois et al. 1956; Swank et al. 1982; Luu and Getsinger 1990) or commercial kits (eg., Sigma Chemical Co., MO).

Chlorophyll *a* Fluorescence

The light harvesting pigment-protein complex of Photosystem II is one of the most stress-sensitive sites in plants (Öquist and Wass 1988), and characteristic changes in amount and kinetics of the energy that escapes as fluorescence from these chlorophylls have been related to the direct and indirect effect of many herbicides (Renger and Schreiber 1986; Bolhàr-Nordenkampf et al. 1989; Miles 1990; MacDonald, Shelling, and Bewick 1993). Transient effect on fluorescence and later recovery have been demonstrated in herbicide-resistant biotypes (Renger and Schreiber 1986), which suggests that variation in fluorescence could differentiate tolerant species in studies of selective herbicide effect.

Modern battery-operated fluorescence meters equipped with microprocessors and fiber-optic cables are compact and relatively inexpensive. Their small size enables them to be used to measure whole-leaf, leaf disk, or canopy fluorescence in the field as well as the laboratory. Rapid, nondestructive methods allow large numbers of measurements to be taken, and the same plants to be monitored over time. Environmental conditions can affect this parameter, and comparisons with untreated controls from the same area are essential.

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Conductivity and Cellular Leakage

The extent of cell membrane disruption caused by herbicide damage can be quantified by measuring the various components leaked into the surrounding medium; electrolytes, which produce an increase in electrical conductivity, are easiest to measure (Vanstone and Stobbe 1977). Excised material such as leaf discs, nodal segments, or apical shoots may be sampled from whole plants and monitored over time in Petri dishes or flasks, using a conductivity meter that reads in 1-μmho/cm increments (Kenyon, Duke, and Vaughn 1985; Duke and Kenyon 1993). To normalize data, total potential leakage can be estimated from tissue that has undergone several freeze-thaw cycles or has been homogenized. Differences in the pattern of electrolyte release have been associated with different herbicide modes of action (Vanstone and Stobbe 1977).

Cytochrome *f*

This compound functions in photosynthetic electron transport, and normally cycles from a photochemically reduced state to a dark-mediated oxidized form. These two cytochrome forms differ in absorbance at 554 nm, with the oxidized form absorbing less, but have the same absorbance at 560 nm. Herbicides that affect photosynthetic function can change electron flow to cytochrome *f*, altering the rate at which the compound is reduced in darkness, or damage the chloroplast to the extent that oxidation/reduction changes are no longer expressed. Compounds that interfere with chloroplast development will suppress the normal oxidative/reductive activity that is initiated as greening takes place. Activity has been measured in whole tissue samples with a dual-wavelength spectrophotometer, as the compound is made to cycle between its oxidized and reduced forms by means of light/dark treatments (Duke et al. 1991; Duke 1993).

Ethylene/Ethane

Rapid elevation in ethylene, with later ethane increase, has been found to result from several herbicides that damage membranes via lipid peroxidation (eg., copper complexes) (Pennazio and Roggero 1991). Increased ethylene has been associated with the synthetic auxin herbicides and discussed as a direct cause of cell death (Hall et al. 1985; Tittle, Goudey, and Spencer 1990; Hall, Alam, and Murr 1993). To collect the evolving gases, excised plant parts may be placed in sealed vessels in the laboratory or field. Ethylene/ethane is then quantified by gas chromatography. This is a relatively rapid quantification procedure, and large numbers of samples can be processed. Careful controls are required because of the numerous environmental and physiological changes that also trigger ethylene production in plants.

Oxidative Enzyme Activity

Activation or increased synthesis of several oxidative enzymes (superoxide dismutase, catalase, peroxidase, and polyphenol oxidase) occurs in many plants during life-cycle changes (eg., Wang, Hong, and Faust 1991) and under abiotic stress (see Scandalios 1993). Rapid or long-term modifications in activity levels of these compounds may be induced either directly from a herbicide's mode of action, as in the case with diquat (Scandalios 1993) or as a result of general metabolic stress. Simple kinetic assays, based on colorimetric reactions between crude plant extract and a suitable substrate, have been used with aquatic plants (eg., Byl and Klaine 1991; Roy, Ihantola, and Hänninen 1992). Species and tissues vary in the constitutive levels of enzyme they express, and the use of different substrates may monitor different isozymic forms of the enzyme (Blume and McClure 1980; Roy, Ihantola, and Hänninen 1992; Sprecher, Stewart, and Brazil 1993b). These considerations need to be taken into account when formulating protocols and sampling schemes.

Oxygen Uptake/Evolution

Monitoring oxygen relationships can reveal direct herbicidal impact on photosynthesis and indirect effects on general metabolic status of the plant. Photosynthetic rate or function is estimated from O_2 evolution in light, and respiration is measured as dark-mediated O_2 consumption. This is readily done with aquatic plants using an O_2 electrode to monitor either plant segments submersed in standard biological oxygen demand (BOD) bottles (Selim et al. 1989; Netherland and Lembi 1992) or whole plants in aquaria (Cassidy and Rodgers 1989). Change in amount of dissolved gas is related to volume, weight of plant tissue present, and incubation period.

Pigments

Certain changes in the colored plant chemicals involved in several key metabolic plant functions are diagnostic for herbicide effect (eg., Duke and Kenyon 1986); others have been correlated to general physiological stress. While pigments can be quantified by HPLC methods, most are readily monitored with spectrophotometric assays of crude extracts of tissue made in organic solvents.

Anthocyanins

These water-soluble pigments are sometimes associated with unfavorable conditions and general stress in plants. They may be unmasked as chlorophyll decreases, being associated in *Potamogeton gramineus* L. with high light

levels (Spencer and Ksander 1990). Fluridone effect often reveals pink to purple coloration as chlorophyll is destroyed.

Carotenoids

These pigments play a role in protecting the integrity of chlorophyll. The ß-carotenes, which comprise the most abundant carotenoids in plants, are usually partitioned into a nonmiscible liquid from crude extracts made in an organic solvent, measured at the absorption maximum, and quantified on the basis of the extinction coefficient of the compound (Sandmann and Böger 1983; Duke, Kenyon, and Paul 1985; Doong, MacDonald, and Shilling 1993).

Chlorophyll

Declining levels of this pigment, caused by indirect herbicide action or decreasing physiological competence, result in loss of ability to produce carbohydrate for growth and maintenance. Total chlorophylls, measured as chlorophyll a and b, can be passively or actively leached into a solvent and quantified spectrophotometrically (Hiscox and Israelstam 1979; Moran and Porath 1980) and are readily analyzed in aquatic plants (Cassidy and Rodgers 1989; Netherland and Lembi 1992; Doong, MacDonald, and Shilling 1993). Frozen samples are able to be held for several months before extraction.

The ratio of chlorophyll to phaeophytin a, a "chlorophyll" molecule without a chelated Mg atom that functions in photosynthetic electron transport, has also been suggested as an indicator of stress. Phaeophytin, as a degradation product of chlorophyll, is relatively low in living chlorophyllous tissue, and this ratio has been used to estimate proportion of living to dead cells (eg., Gaff and Okong'O-Ogola 1971; Marker, Crowther, and Gunn 1980).

Phytoene

Phytoene accumulates when phytoene desaturase inhibition prevents formation of phytofluene and subsequent compounds in the carotenoid biosynthetic pathway. Phytoene can be partitioned along with ß-carotene in crude extracts, and absorbance of this colorless compound is measured in the ultraviolet (UV) range (Sandmann and Böger 1983; Duke, Kenyon, and Paul 1985; Sandmann 1993).

Protein Concentration

Colorimetric tests are commonly used for the rapid and easy estimation of total soluble protein content from crude extracts of plants. Tests based on the analyses of Lowry et al. (1951) and Bradford (1976) are available as packaged kits from commercial sources (eg., Bio-Rad Inc., CA; Sigma Chemical Co.,

MO). Standards of animal proteins (eg., bovine serum albumin and immuno-globulin) are included; these provide an estimation of relative amount of protein present in tested plants rather than an absolute quantification. Total protein determinations can be used to indicate change in protein levels as a measure of metabolic function (eg., Cooley and Foy 1992), as a measure of growth, or as an internal standard against which to normalize other measures such as enzyme activity (Wang, Hong, and Faust 1991).

Tissue Burden

Quantification of the amount of herbicide within plant tissue indicates plant uptake and translocation and gives a measure of bioconcentration. Its importance in studying selective effect on nontarget species is its ability to verify herbicide uptake, quantify translocation, and determine internal herbicide thresholds required for effect. Quantification is available through HPLC or ELISA; cation exchange has also been used to measure diquat in aquatic plants (Cassidy and Rodgers 1989). To indicate amount of herbicide found within specific tissues or at critical intracellular metabolic sites, radioactive- or immuno-labelling are necessary (Sherman and Vaughn 1991; Hinman and Klaine 1992).

5   Conclusions

The technical meeting "Physiological Assessment of Herbicide Stress in Aquatic Plants" provided the CCTT with a valuable introduction to a range of herbicide-related physiological tests, as well as practical guidelines to consider when applying them to CET and selectivity evaluations in submersed plant species. This report has focused on the simpler assays recommended for initial rapid evaluations for herbicide effect. However, numerous other parameters can be considered for metabolic assessment, such as adenylate charge ratios as tests of cell viability (Wiebe and Bancroft 1975; Ciardi et al. 1993) or the use of leaf reflectance to monitor whole-plant response via remote sensing (Gausman and Quisenberry 1990; Carter 1993). Some of the more innovative suggestions made during the meeting look forward to the time when specific biochemical probes will have the potential to reveal DNA-level responses to herbicide effect.

The basic assays described here provide the CCTT with evaluation techniques that are readily incorporated into its herbicide CET and species-selective use studies, facilitating current research goals. Physiological reactions in nontarget species following herbicide exposure can indicate whether response to treatment is direct, indirect, or nonexistent and at what rate or stage an effect is produced. This kind of metabolic data, amassed from aquaria and mesocosm experiments, delimits the treatment tolerances of desirable species and provides a guide to selective herbicide use in the field. These analyses also provide means to evaluate operations-level experiments and monitor the long-term metabolic results of applications in plant communities. In these ways, physiological assessments help to maximize the potential of aquatic herbicides to ameliorate natural ecosystems and restore desirable native plant communities.

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Appendix A
Summary of the Technical Meeting "Physiological Assessment of Herbicide Stress in Aquatic Plants," June 10, 1993

Technical Meeting Schedule

8:15 a.m.   Conference Room, Environmental Processes and Effects Division, U.S. Army Engineer Waterways Experiment Station (WES)

8:30   Introduction to WES and the Chemical Control Technology Team
Dr. Kurt D. Getsinger, Ecosystem and Processes and Effects Branch (EPEB), WES

8:45   Introduction to the Stress Assessment Problem: Overview of herbicide stress/efficacy assessment in aquatic plants; need for additional techniques
Mr. Michael D. Netherland, EPEB, WES

9:00   Herbicide modes of action and consequent opportunities for stress assessment in terrestrial systems
Dr. Stephen O. Duke, U.S. Department of Agriculsture - Agricultural Research Service (USDA-ARS), Southern Weed Science Laboratory/ Stoneville, MS

Reports: Research, Experience, and Ideas

9:30   Conventional evaluation of herbicides for aquatic weed control
Dr. Thai K. Van, USDA-ARS Aquatic Plant Laboratory/Fort Lauderdale, FL

9:45	Physiological indicators of herbicidal efficacy in aquatic and wetland plants Dr. William E. Spencer, Wetlands Resources Branch, WES
10:00	The influence of hydrilla stage-of-development on the mode-of-action of fluridone Dr. Donn G. Shilling, University of Florida/Gainesville
10:20	Break
10:30	Pigment and cell membrane damage as measures of aquatic plant injury due to herbicides Dr. John H. Rodgers, Jr., University of Mississippi/Oxford
10:45	Are changes in pigment levels in *Potamogeton gramineus* an indication of stress? Dr. David F. Spencer, USDA-ARS Aquatic Weed Laboratory/Davis, CA
11:00	Aquatic plant enzyme activity as an indicator of sub-lethal stress Dr. Thomas D. Byl, U.S. Geological Survey/Nashville, TN
11:15	Peroxidase analysis in the CCTT lab Dr. Susan L. Sprecher, EPEB, WES
11:45	Lunch WES Main Cafeteria, Headquarters Building
1:00 p.m.	Tour of CCTT aquaria chambers and laboratory

2:00-4:30	Discussion Session - Dr. Kurt Getsinger, Moderator
	Commentators:
	        Dr. Larry Dyck, Clemson University/Clemson
	        Dr. John D. Madsen, CCTT/Lewisville
	Optimal assessment techniques for specific herbicides
	Summary of findings

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Report Summaries

Speakers provided short abstracts of their presentations, and these were made available to participants prior to the meeting.

Dr. Thomas D. Byl

Aquatic plant enzyme activity as an indicator of sub-lethal stress

Biological changes resulting from exposure to environmental stress such as chronic and acute exposure to chemicals or habitat alteration are useful tools for biomonitoring. This presentation will focus on the use of aquatic plant enzymes as indicators of environmental stress, with special attention to peroxidase enzyme (POD). *Hydrilla verticillata* exposed to increasing concentrations of metals, herbicides or anthracene showed significant increase in whole plant POD activity after a 5 day exposure to 0.01 mg/L of Cu^{2+}, Cd^{2+}, Cr^{6+}, Hg^{2+}, sulfometuron methyl (Oust[R]), and atrazine. There was a significant increase in POD activity after a 5 day exposure to 0.1 mg/L of Se^{4+} and 1.0 mg/L of Mn^{2+}. The POD response was always equal or more sensitive and reliable than growth as an indicator of exposure to these toxicants. There was dose/response relationship between the concentration of toxicant and POD activity. Another enzyme that has been examined after chemical exposure is whole plant dehydrogenase activity. However, the data are often difficult to interpret because after an initial increase in dehydrogenase activity associated with increasing chemical concentration, there would be a decrease in activity. Other enzymes that have received little attention in aquatic plants are catalase

and superoxide dismutase. The concept of using a battery of biochemical markers to extrapolate the type of toxicant deserves further research activity. A better understanding of changes in enzyme activity under field conditions throughout the growing season will eventually allow us to use these enzymes as biomarkers of environmental stress.

Dr. Stephen O. Duke

Herbicide modes of action and consequent opportunities for stress assessment in terrestrial plants

The molecular mechanisms of action of commercial herbicides will be covered briefly. This will be followed by a short discussion of quick, reliable methods of linking terrestrial plant stress to particular herbicides via their mechanism of action. Diquat and glyphosate will be emphasized. The pitfalls of various methods will be pointed out.

Mr. Mike Netherland

Introduction to the stress assessment problem (Summary of comments)

A review of modes of action of available aquatic herbicides was followed by a discussion of the reasons for CCTT interest in determining whether stress measurements can be used to predict efficacy in target populations and evaluate effect on nontarget species. In laboratory, mesocosm and field research, an ability to predict efficacy through change in some parameter would be extremely valuable, but it has not been demonstrated to date. In nontarget populations, stress assessments are expected to be useful in evaluating herbicide selectivity, off-target herbicide movement, herbicide injury for regulatory use, and the presence of terrestrial run-off.

Dr. John H. Rodgers, Jr.

Pigments and cell membrane damage as measures of aquatic plant injury due to herbicides

We have measured pigments (chlorophyll a, pheophytin a, carotenoids, etc.) and pigment ratios to predict the consequences of aquatic plant exposure to herbicides. We have also used several approaches to examine plant cell membrane damage and loss of integrity to assess the consequences of exposures to contact herbicides. Our goal has been to find diagnostic plant biomarkers of stress that are ultimately related to cell and tissue mortality. The

impetus for this research has been use of controlled release herbicides in low concentrations for which symptoms of injury are manifested very slowly.

Dr. Donn G. Shilling

The influence of hydrilla stage-of-development on the mode-of-action of fluridone

The response of hydrilla to fluridone changes depending on the stage of development of the plant at the time of treatment. Hydrilla produces large amounts of shoot tissue very rapidly during vegetative growth, which occurs from spring to summer. During this period, hydrilla shoot tissue is very susceptible to fluridone because of a high demand for new pigments which are used in photosynthesis. Fluridone prevents this pigment formation and therefore growth. Hydrilla reproduces by producing tubers during the fall when shoot growth is extremely slow. Even though hydrilla is growing reproductively, demand for pigments is low because tubers do not contain these pigments and shoot growth is minimal. Consequently, during reproductive growth, shoot tissue is less susceptible to fluridone even though pigment production is blocked. However, these pigments are also used to produce ABA, a plant hormone thought to be involved in hydrilla tuber formation. Therefore, fall fluridone treatments could be used to block reproductive growth even though vegetative growth responses to herbicide treatment would be minimal. Research results dealing with testing these ideas will be presented.

Dr. David Spencer

Are changes in pigment levels in *Potamogeton gramineus* an indication of stress?

Some species of *Potamogeton* may be red or reddish-brown due to the presence of anthocyanins. One hypothesis suggests that *Potamogeton* spp. produce anthocyanins in response to unfavorable conditions such as high light levels, low water temperatures, or nutrient limitation. This hypothesis was tested for a natural population of *Potamogeton gramineus* L. growing in a shallow irrigation canal. Results of nutrient addition experiments suggested that *P. gramineus* in the canal was not nutrient limited. Growing *P. gramineus* at a combination of light and temperatures indicated that the chlorophyll to anthocyanin ratio decreased at high light levels. These results indicate that the reddish-brown appearance of *P. gramineus* is a consequence of a decrease in relative chlorophyll content which unmasks the anthocyanins and may thus not be an indicator of unfavorable conditions.

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Dr. William E. Spencer

Physiological indicators of herbicidal efficacy in aquatic and wetland plants

Selection of physiological indicators of herbicidal efficacy requires that efficacy be explicitly defined, and that indicators adequately predict efficacy. The potential use of C_4 photosynthesis-specific inhibitors for management of aquatic and wetland plants that possess a C_4 photosynthetic pathway is discussed.

Dr. Susan L. Sprecher

Peroxidase analysis in the CCTT lab

Preliminary work has been with various tissues of the aquatic plants milfoil, hydrilla, and egeria, following triclopyr, endothall, and fluridone treatment. Results show that while peroxidase activity is seen to increase with herbicide effect, variation among species, tissues, and enzyme substrates occurs and must be considered in interpreting data and setting up a sampling scheme.

Dr. Thai K. Van

Conventional evaluation of herbicides for aquatic weed control

The USDA Aquatic Plant Management Laboratory in Fort Lauderdale has been actively involved for several years in evaluating herbicides for aquatic use. Chemicals accepted for evaluation are subjected sequentially to a series of efficacy evaluations. Initial evaluations are conducted in controlled-environment laboratory aquaria containing various growth stages of nuisance aquatic plants. Promising chemicals are then evaluated further in large outdoor aquaria and eventually in small field plots. This talk will focus on how this program has evolved over the years, the many problems we have had with it, and the needs for new and more efficient approaches.

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A technical meeting entitled "Physiological Assessment of Herbicide Stress in Aquatic Plants" was held 10 June 1993 at the U.S. Army Engineer Waterways Experiment Station by the Chemical Control Technology Team (CCTT) under the Aquatic Plant Control Research Program. Scientists from universities and government research facilities with common research interests in chemical control of plants and in aquatic weed physiology and ecology were invited to discuss physiological and biochemical methods of verifying and monitoring herbicide effects in submersed aquatic plants. This report summarizes the presentations and discussions by participants on general guidelines for choosing optimal assessment parameters and their suggestions for rapid and simple assays suitable for monitoring physiological changes, whether produced directly by the mode of action of aquatic herbicides or indirectly as a result of loss of metabolic function. Those assays expected to be readily implemented by the CCTT for research assessments of efficacy on target, nontarget, or off-target populations are briefly described.

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